



<110> Hamburger, Joseph
Laban, Avraham

<120> METHODS FOR BULK STABLE PRODUCTION AND EXPRESSION OF FOREIGN GENES
INTO EUKARYOTIC PARASITES FOR IN VIVO PRODUCTION OF DESIRED GENE
PRODUCTS

<130> 01/22115

<140> US 09/857,069

<141> 1999-12-01

<150> US 09/201,850

<151> 1998-12-01

<150> PCT IL 99/00651

<151> 1999-12-01

<160> 9

<170> PatentIn version 3.1

<210> 1

<211> 5356

<212> DNA

<213> Artificial sequence

<220>

<223> GFP-GST recombinant vector sequence

<400> 1
agcttgcatg cctgcaggtc gactctagag gatccccggg taccggtaga aaaaatgagt 60
aaaggagaag aacttttcac tggagttgtc ccaattcttg ttgaattaga tggatgatgtt 120
aatgggcaca aattttctgt cagtggagag ggtgaagggtg atgcaacata cggaaaactt 180
acccttaaatt ttatttgcac tactggaaaa ctacctgttc catggccaac acttgteact 240
actttctctt atggtgttca atgcttttca agataccag atcatatgaa acggcatgac 300

tttttcaaga gtgccatgcc cgaaggttat gtacaggaaa gaactatatt tttcaaagat	360
gacgggaact acaagacacg tgctgaagtc aagtttgaag gtgataccct tgттаataga	420
atcgagttaa aaggtattga ttttaaagaa gatggaaaca ttcttggaca caaattggaa	480
tacaactata actcacacaa tgtatacatc atggcagaca aacaaaagaa tggaaatcaaa	540
gttaacttca aaattagaca caacattgaa gatggaagcg ttcaactagc agaccattat	600
caacaaaata ctccaattgg cgatggccct gtccttttac cagacaacca ttacctgtcc	660
acacaatctg ccctttcgaa agatcccaac gaaaagagag accacatggt ccttcttgag	720
tttgtaacag ctgctgggat tacacatggc atggatgaac tatacaaata gcattcgtag	780
aattctattg cacggtatat ggccaagaaa catcatatga tgggtgaaac agacgaggaa	840
tactatagtg ttgaaaagtt gattggtcag gtgagttgag ccttttattg gttgatggga	900
ttttatgcta ctggagtcct gtaagttcag tttgttgctg tgaataaata aagttcgaat	960
tgataggcga gtggtaacct atgtgtttgtg attactaaaa gtcataagtg tgagggtatcc	1020
caattattag aaatgtgact aagactgagc ttttctgcct attgggatat tacagataag	1080
ttacttattt gtagtatcgt aatagtgttc actgggaacc atctaattca ctctcaaaaa	1140
tactttatcg tgtcttattt tattcatcct cataactcgt ttgctaggag aacagctgtg	1200
cccatagggt agttgacaag ttacttactt ccccatgata cgccttattt taataaccta	1260
ataaaggctc ggttacgtaa cgtccctata gtgcatagca tacctacgaa cattgactcc	1320
caactgatta agtgcggtt gcattttttt ggatgttatc gcacagtaag acaataccat	1380
cctcatctca atgtcaagag gtctttctcc aggcaacaga tccataccac cattacttac	1440
atccatcaga gctgttccca gaatgtcttg ttataaacgg atggattatt tatttatttg	1500
accacataaa tattgcatca aagaggtggg ggatccacta gttctagagc ggccgccacc	1560
gcggtggagc tccagctttt gttcccttta gtgagggtta attgcgcgct tggcgtaatc	1620
atgggcatag ctgtttcctg tgtgaaattg ttatccgctc acaattccac acaacatacg	1680
agccggaagc ataaagtgtg aagcctggg tgccaatga gtgagctaac tcacattaat	1740
tgcgttgccg tcaactgccc ctttccagtc gggaaacctg tcgtgccagc tgcattaatg	1800
aatcggccaa cgcgcgggga gaggcggttt gcgtattggg cgctcttccg cttcctcgct	1860
cactgactcg ctgcgctcgg tcgttcggct gcggcgagcg gtatcagctc actcaaaggc	1920
ggtaatacgg ttatccacag aatcagggga taacgcagga aagaacatgt gagcaaaagg	1980
ccagcaaaag gccaggaacc gtaaaaaggc cgcgttgctg gcgtttttcc ataggctccg	2040
ccccctgac gagcatcaca aaaatcgacg ctcaagtcag aggtggcgaa accgacagga	2100
ctataaagat accaggcgtt tccccctgga agctccctcg tgcgctctcc tgttccgacc	2160
ctgccgctta ccggatacct gtccgccttt ctcccttcgg gaagcgtggc gctttctcat	2220

agctcacgct gtaggtatct cagttcgggtg taggtcggtc gctccaagct gggctgtgtg	2280
cacgaccccc cgttcagccc gaccgctgcg ccttatccgg taactatcgt cttgagtcca	2340
acccggtaag acacgactta tcgccactgg cagcagccac tggtaacagg attagcagag	2400
cgaggtatgt aggcgggtgt acagagttct tgaagtgggt gcctaactac ggctacacta	2460
gaaggacagt atttggtatc tgcgctctgc tgaagccagt taccttcgga aaaagagttg	2520
gtagctcttg atccggcaaa caaaccaccg ctggtagcgg tggttttttt gtttgcaagc	2580
agcagattac gcgcagaaaa aaaggatctc aagaagatcc tttgatcttt tctacggggt	2640
ctgacgctca gtggaacgaa aactcacgtt aagggatttt ggtcatgaga ttatcaaaaa	2700
ggatcttcac ctagatcctt ttaaattaaa aatgaagttt taaatcaatc taaagtatat	2760
atgagtaaac ttggtctgac agttaccaat gcttaatcag tgaggcacct atctcagcga	2820
tctgtctatt tcgttcatcc atagttgcct gactccccgt cgtgtagata ctacgatacg	2880
ggagggctta ccatctggcc ccagtgtctc aatgataccg cgagaccac gtcaccggc	2940
tccagattta tcagcaataa accagccagc cggaagggcc gagcgcagaa gtggtcctgc	3000
aactttatcc gcctccatcc agtctattaa ttgttgccgg gaagctagag taagtagttc	3060
gccagttaat agtttgcgca acgttgttgc catttgctaca ggcacgtgg tgtcacgctc	3120
gtcgtttggt atggcttcat tcagctccgg ttcccaacga tcaaggcgag ttacatgac	3180
ccccatgttg tgcaaaaaag cggttagctc cttcggctct ccgatcgttg tcagaagtaa	3240
gttgccgca gtgttatcac tcatggttat ggcagcactg cataattctc ttactgtcat	3300
gccatccgta agatgctttt ctgtgactgg tgagtactca accaagtcac tctgagaata	3360
gtgtatgagg cgaccgagtt gctcttgccc gggtcaata cgggataata ccgcgccaca	3420
tagcagaact ttaaaagtgc tcatcattgg aaaacgttct tcggggcgaa aactctcaag	3480
gatcttaccg ctgttgagat ccagttcgat gtaaccact cgtgcacca actgatcttc	3540
agcatctttt actttcacca gcgtttctgg gtgagcaaaa acaggaaggc aaaatgccgc	3600
aaaaaagga ataagggcga cacggaatg ttgaatactc atactcttc tttttcaata	3660
ttattgaagc atttatcagg gttattgtct catgagcgga tacatatttg aatgtattta	3720
gaaaaataaa caaatagggg ttccgcgcac atttccccga aaagtgccac ctgacgcgcc	3780
ctgtagcggc gcattaagcg cggcgggtgt ggtggttacg cgcagcgtga ccgctacact	3840
tgccagcgcc ctagcgccc ctcctttcgc tttcttcctc tcctttctcg ccacgttcgc	3900
cggctttccc cgtcaagctc taaatcgggg gctcccttta gggttccgat ttagtgcttt	3960
acggcacctc gaccccaaaa aacttgatta gggtagtggt tcacgtagtg ggccatcgcc	4020
ctgatagacg gtttttcgcc ctttgacgtt ggagtccacg ttctttaata gtggactctt	4080
gttccaaact ggaacaacac tcaaccctat ctcggtctat tcttttgatt tataagggat	4140
tttgccgatt tcggcctatt ggttaaaaaa tgagctgatt taacaaaaat ttaacgcgaa	4200

```

ttttaacaaa atattaacgc ttacaatttc cattcgccat tcaggctgcg caactgttgg 4260
gaagggcgat cgggtgcgggc ctcttcgcta ttacgccagc tggcgaaagg gggatgtgct 4320
gcaaggcgat taagttgggt aacgccaggg ttttcccagt cacgacgttg taaaacgacg 4380
gccagtgagc gcgcgtaata cgactcacta tagggcgaaat tgggtaccgg gccccccctc 4440
gaggtcgacg gtatcgataa gcttgattca tcgagaacgg tttacatgtt caatgaatcg 4500
agtcaaattt gtctgcttaa tttttatttg tcaactcttc acagccaatg aggcaactcaa 4560
taaacagcga atagaaatga aatatttaca gttaaaatca agagttacac tattggccga 4620
tctgtttact aaatgactat ttaatagtc cagctcagat agttagcact gttttgtcta 4680
tttgcaagat ggctggcgag catatcaagg tgggttggtg tggttgttcg tcattgtgtt 4740
gcttggtaaa ttttcgagtg ttgttaaggt tcctaattgc agcatttagc ggaataaata 4800
ttattccgct atatgaaggt aaatactgag tccatgttaa ttgcacatta gatccgtaca 4860
tatatttggc tgtgattttc cacgtcatca ggtcagtaag ttacgtgttc aactggggtt 4920
ctgatataat gcacaaccat tatagtcaca tagtggtcac cgattatgtg ttaggtgtag 4980
catttcaggc gatgttatag tttgtctgtg gaattgttac gagaacttag gggattctag 5040
agcgtgaatc catctagtcc atatctcaga gacatgcgtc catggaacag ttatttatta 5100
tgtgatgttt tgtctgaagt ggtttttttc aagcgtaaat tgatgtgaaa cacagctgta 5160
atctctatag gttatctatt ttgacggacg cggacgtgct gaatcgattc ggatgactct 5220
tgtggcagct ggtgtagact acgaagatga gagaattagt ttccaagatt ggccaaaaat 5280
caaaccaact attccaggcg gacgattgcc tgcagtgaaa gtcactgatg atcatgggca 5340
cgtgaaatgg atgtta 5356

```

<210> 2

<211> 6494

<212> DNA

<213> Artificial sequence

<220>

<223> GFP-Sm1-7 fusion, vector sequence

<400> 2

```

gatctgaatc cgaccaaccg ttctatgaaa atcgttgat ctccgaaacc actggacgga 60
tttttatgat gtttgtttta gattatttgc gagagcgtgg gcgttaatat aaaacaagaa 120
tgatctgaat ccgaccaacc gttctatgaa aatcgttgta tctccgaaac cactggacgg 180
atttttatga tgtttgtttt agattatttg cgagagcgtg ggcgttaata taaaacaaga 240

```

atgatctgaa tccgaccaac cgttctatga aaatcgttgt atctccgaaa ccactggacg	300
gatttttatg atgtttgttt tagattatgt gcgagagcgt gggcggttaat ataaaaacaag	360
aatcatctca atcccatcag ccgttctatg aaaatcgttg tatctccgaa accactggac	420
ggatttttat gatgtttgtt ttagattatt tgcgagagcg tgggcgttaa tataaaacaa	480
gaatgatctg aacacgggtg tttttctgtt cagcttatgc aactttaaaa ttcgatgggt	540
cgtctcaacg aaatttgtat tgctttgtcg aggtcgacgg tatcgataag cttgattcat	600
cgagaacggg ttacatgttc aatgaatcga gtcaaatttg tctgcttaat ttttattggg	660
cactctttca cagccaatga ggcactcaat aaacagcgaa tagaaatgaa atatttacag	720
ttaaaatcaa gagttacact attggccgat ctgtttacta aatgactatt taatagtcac	780
agctcagata gttagcactg ttttgtctat ttgcaagatg gctggcgagc atatcaagg	840
gggttggtgt gggtgttcgt cattgtgttg cttggtaaat tttcgagtgt tgtaagggt	900
cctaattgca gcatttagcg gaataaatat tattccgcta tatgaaggta aatactgagt	960
ccatgttaat tgcacattag atccgtacat atatttggct gtgattttcc acgtcatcag	1020
gtcagtaagt tacgtgttca cactgggttc tgatataatg cacaaccatt atagtcacat	1080
agtggtcacc gattatgtgt taggtgtagc atttcaggcg atgttatagt ttgtctgtgg	1140
aattgttacg agaacttagg ggattctaga gcgtgaatcc atctagtcca tatctcagag	1200
acatgcgtcc atggaacagt tatttattat gtgatgtttt gtctgaagtg gtttttttca	1260
agcgtaaatt gatgtgaaac acagctgtaa tttctatagg ttatctatgt tgacggacgc	1320
ggacgtgctg aatcgattcg gatgactcct gtggcagctg gtgtagacta cgaagatgag	1380
agaattagtt tccaagattg gccaaaaatc aaaccaacta ttccaggcgg acgattgcct	1440
gcagtgaag tcaactgatg tcatgggcac gtgaaatgga tgtaagctt gcatgcctgc	1500
aggtcgactc tagaggatcc ccgggtaccg gtagaaaaaa tgagtaaagg agaagaactt	1560
ttcactggag ttgtcccaat tcttgttgaa ttagatgggt atgttaatgg gcacaaattt	1620
tctgtcagtg gagagggtga aggtgatgca acatacggaa aacttaccct taaatttatt	1680
tgcactactg gaaaactacc tggtccatgg ccaacacttg tcaactactt ctcttatgg	1740
gttcaatgct tttcaagata ccagatcat atgaaacggc atgacttttt caagagtgcc	1800
atgcccgaag gttatgtaca ggaaagaact atatttttca aagatgacgg gaactacaag	1860
acacgtgctg aagtcaagtt tgaagggtgat acccttggtt atagaatcga gttaaaagg	1920
attgatttta aagaagatgg aaacattcct ggacacaaat tggaatacaa ctataactca	1980
cacaatgtat acatcatggc agacaaacaa aagaatggaa tcaaagttaa cttcaaaatt	2040
agacacaaca ttgaagatgg aagcgttcaa ctacgagacc attatcaaca aaatactcca	2100
attggcgatg gccctgtcct tttaccagac aaccattacc tgtccacaca atctgcctt	2160
tcgaaagatc ccaacgaaaa gagagaccac atggctcttc ttgagtttgt aacagctgct	2220

gggattacac atggcatgga tgaactatac aaatagcatt cgtagaattc tattgcacgg 2280
 tatatggcga agaaacatca tatgatgggt gaaacagacg aggaatacta tagtggtgaa 2340
 aagttgattg gtcaggtgag ttgagccttt tattggttga tgggatttta tgctactgga 2400
 gtcctgtaag ttcagtttgt tgcgttgaat aaataaagtt cgaattgata ggcgagtggg 2460
 aacctatgtg ttgtgattac taaaagtcac aagtgtgagg tatcccaatt attagaaatg 2520
 tgactaagac tgagcttttc tgcctattgg gatattacag ataagttact tatttgtagt 2580
 atcgtaatag tgttcactgg gaaccatcta attcactctc aaaaataactt tatcgtgtct 2640
 tattttattc atcctcataa ctggtttgct aggagaacag ctgtgcccac aggttagttg 2700
 acaagttact tacttcccca tgatacgctt tattttaata acctaataaa ggctcgggta 2760
 cgtaacgtcc ctatagtga tagcatacct acgaacattg actcccaact gattaagtgc 2820
 ggattgcatt tttttggatg ttatcgaca gtaagacaat accatcctca tctcaatgtc 2880
 aagaggtctt tctccaggca acagatccat accaccatta cttacatcca tcagagctgt 2940
 tcccagaatg tcttggtata aacggatgga ttattttattt atttgaccac ataaatattg 3000
 catcaaagag gtgggggatc cactagttct agagcggccg ccaccgcggt ggaggatctg 3060
 aatccgacca accgttctat gaaaatcgtt gtatctccga aaccactgga cggattttta 3120
 tgatgtttgt tttagattat ttgcgagagc gtgggcgtta atataaaaca agaagatctt 3180
 gaatccgacc aaccgttcta tgaaaatcgt tgtatctccg aaaccactgg acggattttt 3240
 atgatgtttg ttttagatta tttgcgagag cgtgggcgtt aatataaaac aagaatgatc 3300
 tgaatccgac caaccgttct atgaaaatcg ttgtatctcc gaaaccactg gacggatttt 3360
 tatgatgttt gtttttagatt atttgcgaga gcgtgggcgt taatataaaa caagaatcat 3420
 ctcaatccca tcagccgttc tatgaaaatc gttgtatctc cgaaaccact ggacggattt 3480
 ttatgatgtt tgtttttagat tatttgcgag agcgtgggcg ttaatataaa acaagaatga 3540
 tctgaacacg ggtgtttttc tggtcagctt atgcaacttt aaaattcgat gggtcgtctc 3600
 aacgaaatth gtattgcttt gctccagctt ttgttccctt tagtgagggt taattgcgcg 3660
 cttggcgtaa tcatggtcat agctgtttcc tgtgtgaaat tgttatccgc tcacaattcc 3720
 acacaacata cgagccggaa gcataaagtg taaagcctgg ggtgcctaät gagtgagcta 3780
 actcacatta attgcgttgc gtcactgcc cgctttccag tcgggaaacc tgcgtgcca 3840
 gctgcattaa tgaatcgcc aacgcgcggg gagaggcggg ttgcgtattg ggcgctcttc 3900
 cgcttcctcg ctactgact cgctgcgctc ggctgttcgg ctgcggcgag cggtatcagc 3960
 tcaactcaaag gcggtataac ggttatccac agaatcaggg gataacgcag gaaagaacat 4020
 gtgagcaaaa ggccagcaaa aggccaggaa ccgtaaaaag gccgcgttgc tggcgttttt 4080
 ccataggctc cgccccctg acgagcatca caaaaatcga cgctcaagtc agaggtggcg 4140

aaaccgacag gactataaag ataccaggcg tttcccctg gaagctccct cgtgcgctct	4200
cctgttccga ccctgccgct taccggatac ctgtccgcct ttctcccttc gggaagcggtg	4260
gcgctttctc atagctcacg ctgtaggatc ctcagttcgg tgtaggtcgt tcgctccaag	4320
ctgggctgtg tgcacgaccc cccgttcagc ccgaccgctg cgccttatcc ggtaactatc	4380
gtcttgagtc caacccggtg agacacgact tatcgccact ggcagcagcc actggtaaca	4440
ggattagcag agcgaggatg gtaggcgggtg ctacagagtt cttgaagtgg tggcctaact	4500
acggctacac tagaaggaca gtatttggtg tctgcgctct gctgaagcca gttaccttcg	4560
gaaaaagagt tggtagctct tgatccggca aacaaaccac cgctggtagc ggtggttttt	4620
ttgtttgcaa gcagcagatt acgcgcagaa aaaaaggatc tcaagaagat cctttgatct	4680
tttctacggg gtctgacgct cagtggaaacg aaaactcacg ttaagggatt ttggtcatga	4740
gattatcaaa aaggatcttc acctagatcc ttttaaatta aaaatgaagt tttaaatcaa	4800
tctaaagtat atatgagtaa acttgggtctg acagttacca atgcttaatc agtgaggcac	4860
ctatctcagc gatctgtcta tttcgttcat ccatagttgc ctgactcccc gtcgtgtaga	4920
tactacgata cgggagggct taccatctgg ccccgagtgt gcaatgatac cgcgagaccc	4980
acgctcaccg gctccagatt tatcagcaat aaaccagcca gccggaaggg ccgagcgcag	5040
aagtggtcct gcaactttat ccgcctccat ccagttctatt aattgttgcc gggaagctag	5100
agtaagtagt tcgccagtta atagtttgcg caacgttggt gccattgcta caggcatcgt	5160
ggtgtcacgc tcgtcgtttg gtatggcttc attcagctcc ggttcccaac gatcaaggcg	5220
agttacatga tccccatgt tgtgcaaaaa agcggttagc tccttcggtc ctccgatcgt	5280
tgtcagaagt aagttggccg cagtgttatc actcatggtt atggcagcac tgcataatc	5340
tcttactgtc atgccatccg taagatgctt ttctgtgact ggtgagtact caaccaagtc	5400
attctgagaa tagtgatgac ggcgaccgag ttgctcttgc ccggcgtcaa tacgggataa	5460
taccgcgcca catagcagaa ctttaaaagt gtcatcatt ggaaaacgtt cttcggggcg	5520
aaaactctca aggatcttac cgctgttgag atccagttcg atgtaacca ctctgtcacc	5580
caactgatct tcagcatctt ttactttcac cagcgtttct gggtagcaa aaacaggaag	5640
gcaaaatgcc gcaaaaaagg gaataagggc gacacggaaa tgttgaatac tcatactctt	5700
cctttttcaa tattattgaa gcatttatca gggttattgt ctcatgagcg gatacatatt	5760
tgaatgtatt tagaaaaata aacaaatagg ggttcgcgc acatttcccc gaaaagtgcc	5820
acctgacgcg ccctgtagcg gcgcattaag cgcggcggtg gtggtggtta cgcgcagcgt	5880
gaccgctaca cttgccagcg ccctagcgcc cgctccttcc gctttcttcc cttcctttct	5940
cgccacgttc gccggctttc cccgtcaagc tctaaatcgg gggctccctt tagggttccg	6000
athtagtgct ttacggcacc tcgaccccaa aaaacttgat tagggtgatg gttcacgtag	6060
tgggccatcg ccctgataga cggtttttcg ccctttgacg ttggagtcca cgttctttaa	6120

tagtggactc ttgttccaaa ctggaacaac actcaaccct atctcggctct attcttttga 6180
 tttataaggg attttgccga tttcggccta ttggttaaaa aatgagctga ttttaacaaaa 6240
 atttaacgcg aattttaaca aaatattaac gcttacaatt tccattcgcc attcaggctg 6300
 cgcaactggt gggaagggcg atcgggtcgg gcctcttcgc tattacgcca gctggcgaaa 6360
 gggggatgtg ctgcaaggcg attaagttgg gtaacgccag ggttttccca gtcacgacgt 6420
 tgtaaaacga cggccagtga gcgcgcgtaa tacgactcac tatagggcga attgggtacc 6480
 gggccccccc tcga 6494

<210> 3

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 3

catcgagaac gggtttacatg ttca

24

<210> 4

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 4

gcagcctcct cacatgctcc a

21

<210> 5

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 5
atgagtaaag gagaagaact tttc

24

<210> 6

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 6
tttgtatagt tcatccatgc c

21

<210> 7

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 7
tctcccatga tgtatacatt atgt

24

<210> 8

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Synthetic oligonucleotide

<400> 8
tctccatcga agggatcatca cg

22

<210> 9

<211> 121

<212> DNA

<213> Schistosoma haematobium

<400> 9

gatctcacct atcagacgaa acaaagaaaa ttttaaaatt gttgggtggaa gtgcctgttt 60

cgcaatatct ccggaatggt tggtcgtatc gttgtgaaaa ttgtttcata ttattggtga 120

c 121